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Exposure to Per- and Polyfluoroalkyl Substances and Mortality in U.S. Adults: A Population-Based Cohort Study

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Background: Per- and polyfluoroalkyl substances (PFAS) are widespread environmental contaminants associated with diseases such as cancer and dyslipidemia. However, few studies have investigated the association between PFAS mixture exposure and mortality in general populations.

Objectives: This study aimed to explore the association between PFAS mixture, perfluorooctanoic acid (PFOA), and perfluorooctane sulfonic acid (PFOS) and mortality in U.S. adults by a nationally representative cohort.

Methods: Adults ≥18 years of age who were enrolled in the National Health and Nutrition Examination Survey (NHANES) (1999–2014) were included in our study. Baseline serum concentrations of seven PFAS were measured and individuals were followed up to 31 December 2015. Hazard ratios (HRs) and confidence intervals (CIs) were estimated using Cox proportional hazards models. Association between PFAS mixture exposure and mortality was analyzed using the *k*-means method by clustering PFAS mixtures into subgroups. Association between PFOA/PFOS exposure and mortality was subsequently analyzed in both continuous and categorical models.

Results: During the follow-up period, 1,251 participants died. In the mixture analysis, the *k*-means algorithm clustered participants into low-, medium-, and high-exposure groups. Compared with the low-exposure group, participants in the high-exposure group showed significantly higher risks for all-cause mortality (HR = 1.38; 95% CI: 1.07, 1.80), heart disease mortality (HR = 1.58; 95% CI: 1.05, 2.51), and cancer mortality (HR = 1.70; 95% CI: 1.08, 2.84). In single PFAS analysis, PFOS was found to be positively associated with all-cause mortality (third vs. first tertile HR = 1.57; 95% CI: 1.22, 2.07), heart disease mortality (third vs. first tertile HR = 1.65; 95% CI: 1.09, 2.57), and cancer mortality (third vs. first tertile HR = 1.75; 95% CI: 1.10, 2.83), whereas PFOA exposure had no significant association with mortality. Assuming the observed association is causal, the number of deaths associated with PFOS exposure (≥17.1 vs. <7.9 ng/mL) was ∼382,000 (95% CI: 176,000, 588,000) annually between 1999 and 2015, and it decreased to 69,000 (95% CI: 28,000, 119,000) annually between 2015 and 2018. The association between PFOS and mortality was stronger among women and people without diabetes.

Discussion: We observed a positive association between PFAS mixture exposure and mortality among U.S. adults. Limitations of this study include the potential for unmeasured confounding, selection bias, a relatively small number of deaths, and only measuring PFAS at one point in time. Further studies with serial measures of PFAS concentrations and longer follow-ups are necessary to elucidate the association between PFAS and mortality from specific causes. https://doi.org/10.1289/EHP10393

Introduction

Per- and polyfluoroalkyl substances (PFAS) have been produced since the 1950s and are widely used in multiple commercial applications, including in surfactants, lubricants, paints, polishes, food packaging, and fire-retarding foams.[1] As a consequence of the wide use and their resulting emissions, several of these PFAS became ubiquitous contaminants that can be easily found in both humans and wildlife.[2] Diseases reported to be associated with PFAS exposure include cancer,[3] dyslipidemia,[4] ovarian disorders,[5] thyroid dysfunction,[6] and impaired fetal growth.[7] Humans are exposed to PFAS mainly through dietary intake.[8] Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) are of particular concern because of their persistent characteristics, wide distribution, and bioaccumulative properties.[9] Although PFOA and PFOS were gradually phased out globally from 2000 to 2015,[9] these two chemicals can still be found in the surface water and soils globally.[10–12] A cross-sectional study conducted in Washington State in 2019 revealed that PFOA and PFOS can be detected in 86% and 100% breast milk samples from breastfeeding women, with median levels of 30.4 and 13.9 ng/L, respectively.[13] These results indicate that PFOA and PFOS remain contaminants of concern. Despite the extensive studies that have explored the associations between PFOA/PFOS exposure and human health outcomes, with some consistent evidence reported on cancer, hypercholesterolemia, and liver and immune dysfunction,[3,14] no consistent conclusion has been reached yet on their links with mortality in general populations. In addition, few studies have investigated the associations between exposure to PFAS mixtures and health outcomes.[15] Considering that PFAS usually exist as a mixture,[16] statistical models stratifying the study population-based PFAS mixture may provide further insight into the potential adverse effects of PFAS exposure on health.

Using the follow-up data from the 1999–2014 National Health and Nutrition Examination Survey (NHANES), we conducted a population-based prospective study to explore the relationships between exposure to PFAS mixtures and human all-cause, heart disease, and cancer mortality, using an unsupervised clustering (*k*-means) method based on the concentrations of seven PFAS in serum. We also analyzed the associations between exposure of

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two representative PFAS—PFOA and PFOS—and mortality in U.S. adults, and estimated the number of deaths in U.S. adults associated with PFOS exposure.

Methods

Study Population

NHANES is a continuously conducted, nationally representative, and complex cross-sectional survey designed to monitor the health and nutritional status of the noninstitutionalized U.S. population.[17] We included individuals who were ≥18 years of age at baseline (mortality data on participants <18 years of age are unavailable for public release). The NHANES protocol is in compliance with the Department of Health and Human Services Policy for the Protection of Human Research Subjects, and informed consent was obtained from participants who had reached the age of maturity in their state (usually ≥18 years of age).[18,19]

Procedures

Baseline information was collected from 1999 to 2014, when individuals participated in a household interview and a medical examination, during which they provided blood samples and basic information such as sex, age, ethnic origin, household income, education, physical activity, alcohol consumption, smoking status, and medical history (including diseases, such as hypertension and diabetes, and the use of prescription medications). During the medical examination, weight, height, and blood pressure were measured. Diet data were derived from food frequency questionnaires obtained from in-person interviews and telephone dietary interviews.

Concentrations of PFAS, total cholesterol, cotinine, and creatinine were measured in blood samples collected during the medical examination. The detailed specimen collection and processing instructions are reported in the NHANES Laboratory/Medical Technologists Procedures Manual.[20] PFAS were quantified in serum by solid-phase extraction–high-performance liquid chromatography–turbo-ionspray ionization–tandem mass spectrometry (SPE-HPLC-TCI-MS/MS). The limit of quantification (LOQ) was 0.1 ng/mL for both PFOA and PFOS. The LOQs for other PFAS are presented in Table S1. Samples with PFAS concentrations below the LOQ were substituted with the value of the LOQ divided by the square root of 2.

A detailed description of mortality linkage methods has been reported previously.[21] Briefly, based on a series of identifiers, such as social security number and date of birth, the National Center for Health Statistics (NCHS) linked participants in NHANES 1999–2014 to the underlying causes of death in the National Death Index using probabilistic matching criteria. Participants were followed up to 31 December 2015. If a match was not made with the National Death Index, that person was assumed to be alive as of that date. For the 1999–2006 NHANES, nine cause-specific death categories were included in the public-use linked mortality files, whereas for the 2007–2014 NHANES, only two cause-specific death categories (heart disease and cancer) were included in the public-use linked mortality files owing to the short follow-up time and small sample sizes for the other cause-specific death categories.

Statistical Analysis

Statistical analyses were conducted using SAS (version 9.4; SAS Institute, Inc.), and $p < 0.05$ was considered as statistically significant. Results regarding percentiles, means, point estimates, and risk estimates were adjusted using the provided specific sample weights to account for the complex survey design of the NHANES and to make these data representative of the U.S. civilian noninstitutionalized resident population. According to the weight selection guideline,[22] mobile examination center (MEC) weights of subsamples for PFAS detection were used in this study. The SAS code for the weight adjustment procedure is provided in Table S2.

We calculated Pearson correlation coefficients to evaluate the correlations among serum concentrations of seven PFAS. For PFAS mixture analysis, we applied the *k*-means method to categorize participants into different clusters based on the log-transformed serum concentrations of seven PFAS. The *k*-means algorithm is a non–model-based method that can be used to categorize mixture data.[23,24] The *k*-means algorithm constructs clusters so that the squared Euclidean distance between the row vector for any object and the centroid vector of its respective cluster is at least as small as the distances to the centroids of the remaining clusters.[25] The optimal number of clusters was determined by the elbow method,[25] and the subgroups were dimensionality reduced and visualized by t-Distributed Stochastic Neighbor Embedding (t-SNE). We assessed the relationship between PFAS coexposure and population mortality by a categorical model based on the clusters obtained from the *k*-means algorithm. To clarify the association between single PFAS exposure and human mortality, PFOA and PFOS were selected for further analysis given that they had the highest detection rates and concentrations in our study population (Table S3) and are also the two most studied PFAS traditionally. Five-knot restricted cubic splines were fitted to estimate exposure–response curves of serum PFOA/PFOS concentrations and all-cause, heart disease, and cancer mortality. Categorical analysis on PFOA/PFOS was also conducted by categorizing participants into three groups based on the tertiles of serum concentrations.

We calculated hazard ratios (HRs) and confidence intervals (CIs) for PFAS mixture/PFOA/PFOS exposure using Cox proportional hazards models. The proportional hazards assumption was evaluated by Schoenfeld residuals,[26] and none of the models violated the assumption. As defined by the NHANES protocol, participant's survival was the time between the medical examination and the date of death, the participant's 90th birthday, or 31 December 2015, whichever came first.[21] Variables widely recognized as potential confounders for mortality were evaluated: age (continuous), sex (male and female), race/ethnicity [self-reported as non-Hispanic White, non-Hispanic Black, Mexican American, and other (including other Hispanic, other race, and multiracial)], education (with or without high school education), household income (<\$20,000 or ≥\$20,000/y), smoking status (never, former smoker, or current smoker), alcohol consumption (<1 d/wk or ≥1 d/wk), physical activity (0–14 times or ≥15 times/month), hypertension (defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg), diabetes (self-reported yes or no), and body mass index (BMI) [normal weight (<25.0 kg/m^2), overweight (25.0–29.9 kg/m^2), and obesity (≥30.0 kg/m^2)]. Blood pressure was measured three to four times for each participant, and the average value (in millimeters mercury) was calculated by excluding the first reading and using the remaining measures. Dietary habits were assessed by the Healthy Eating Index derived from food frequency questionnaires and scored on a scale from 1 to 100[27] and were categorized into tertiles. We estimated creatinine clearance rate (Ccr, in milliliters per minute) using serum creatinine levels based on the formula: $\text{Ccr (mL/min)} = (140\text{-Age [y]} \times \text{Body weight [kg]})/(72 \times \text{Scr [mg/dL]})$. Ccr for female participants is multiplied by 0.85 based on the results of the above formula. Finally, we included serum total cholesterol concentration (in milligrams per deciliter) and serum cotinine amount (in nanograms per milliliter) as continuous

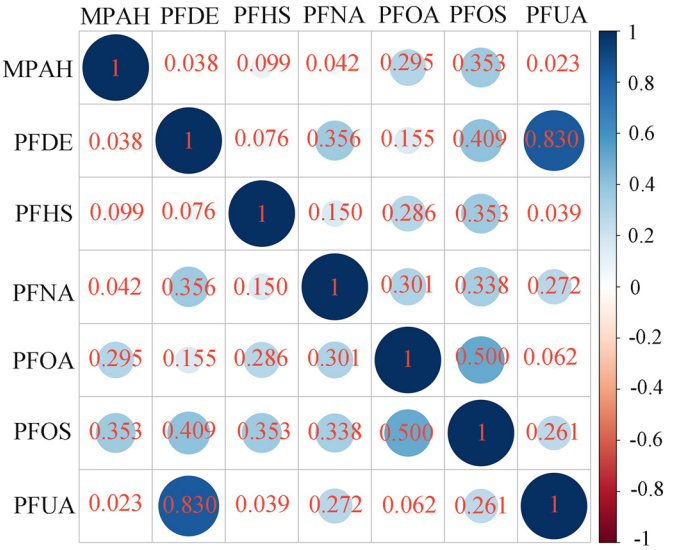

Figure 1. Pearson correlation analysis on serum concentrations of seven PFAS in NHANES participants (1999–2014, $n = 11{,}747$). The most significant correlation was observed between serum concentrations of PFUA and PFDE (correlation = 0.830). Serum concentrations of other PFAS showed weak-to-moderate correlations ($0.02 <$ correlation ≤ 0.50). Samples with serum PFAS concentrations below the LOQ were substituted with the value of the LOQ divided by the square root of 2. Note: LOQ, limit of quantification; MPAH, 2-(*N*-methyl-perfluorooctane sulfonamido) acetic acid; NHANES, National Health and Nutrition Examination Survey; PFAS, per- and polyfluoroalkyl substances; PFDE, perfluorodecanoic acid; PFHS, perfluorohexane sulfonate acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFUA, perfluoroundecanoic acid.

measures. Missing data on covariates were processed using the multiple imputation algorithm.[28] Only variables that were significantly associated with the exposure levels of PFAS mixture/PFOA/PFOS were chosen as confounders. Associations between confounders and mortality were also estimated using Cox proportional hazards models.

We calculated population attributable fractions for PFOS exposure using the method proposed by Levin to estimate the proportional reduction in mortality from 1999 to 2015 that would occur if the recorded amounts of PFOS in serum in the entire U.S. population ≥18 years of age reduced from ≥17.1 ng/mL (third tertile) to <7.9 ng/mL (first tertile).[29,30] CIs for population attributable fractions were calculated using a substitution method proposed by Daly.[31] Numbers of deaths were calculated based on the average annual number of deaths from all causes, heart disease, and cancer from 1999 to 2015, which are available from the NCHS National Vital Statistics System website.[32] To estimate the number of deaths associated with PFOS exposure in recent years, we calculated the proportion of the population with serum PFOS concentration at ≥17.1 ng/mL and the proportion of the population with serum PFOS <7.9 ng/mL using data of NHANES 2015–2018. The population attributable fractions were estimated by comparison of the HR in the highly exposed population (serum PFOS ≥17.1 ng/mL) with the HR in the lowly exposed population (serum PFOS <7.9 ng/mL) weighted by their proportions.[33]

Sensitivity analyses were conducted by making several adjustments on confounders, such as age, hypertension, dietary habits, and smoking status. These adjustments were characterizing serum concentrations of PFAS as continuous variables instead of the three-categorized PFAS mixture; characterizing age as a categorical variable (<50 or ≥50 y) instead of as a continuous variable; adjusting for hypertension status by defining hypertension as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or use of anti-hypertension drugs; adjusting for continuous systolic blood pressure and diastolic blood pressure instead of categorical measures; adjusting for continuous healthy eating index instead of categorical evaluations; adjusting for amount and duration of smoking among past and current smokers instead of simply classifying smoking history as never, former, and current smoker. Considering that participants who die might go through a period of illness before death, during which whose intake of food (and consequently, PFAS) might decrease, we also performed a sensitivity analysis excluding subjects who died within a year of blood draw. Finally, using a log-likelihood ratio test, we assessed potential effect modification of key characteristics, including sex, age, race/ethnicity, hypertension, smoking status, diabetes, and obesity, on the relation between PFOS exposure and all-cause, heart disease, and cancer mortality.

Results

Correlations among Serum Concentrations of Seven PFAS

After excluding three PFAS [2-(*N*-ethyl-perfluorooctane sulfonamido) acetic acid (EPAH), perfluorooctane sulfonamide (PFSA), and perfluorobutane sulfonic acid (PFBS)], whose serum concentrations were not measured in one or more NHANES cycles from 1999 to 2014, and two PFAS [perfluorododecanoic acid (PFDO)

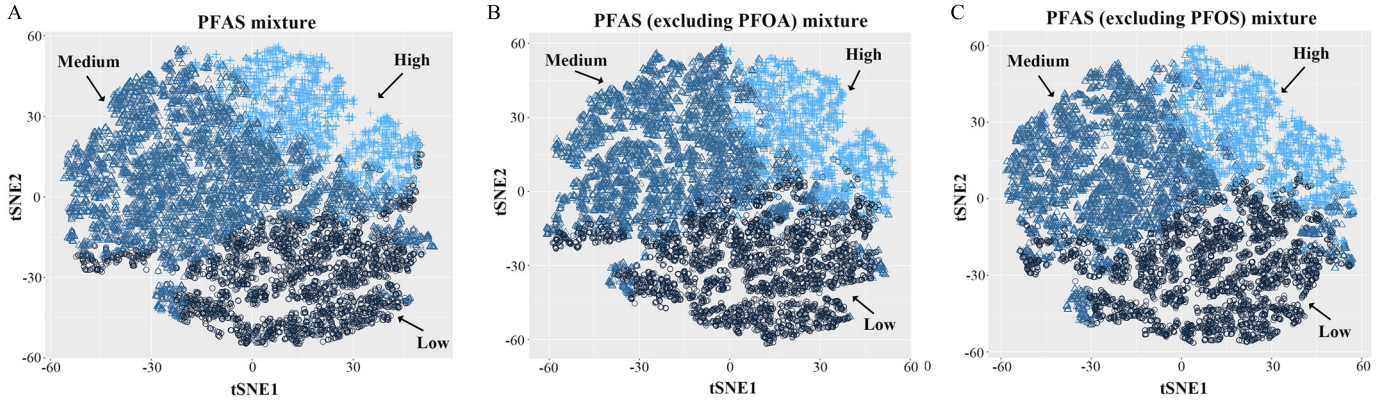

Figure 2. t-SNE visualization of *k*-means clustering of NHANES participants (1999–2014, $n = 11{,}747$) based on serum concentrations of PFAS. Three subgroups (low-, medium-, and high-exposure groups) were obtained based on the mixture of (A) total PFAS, (B) PFAS excluding PFOA, and (C) PFAS excluding PFOS. Samples with serum PFAS concentrations below the LOQ were substituted with the value of the LOQ divided by the square root of 2. Note: LOQ, limit of quantification; NHANES, National Health and Nutrition Examination Survey; PFAS, per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; t-SNE, t-Distributed Stochastic Neighbor Embedding.

Table 1. Comparison of serum PFAS concentrations of the NHANES participants (1999–2014, $n = 11{,}747$) among three subgroups divided by *k*-means method.

PFAS mixtures		Serum concentrations (GM ± SE and medians and quartiles) of PFAS in subgroups divided by *k*-means method (ng/mL)			*p*-Value (medium vs. low)	*p*-Value (high vs. medium)
		Low	Medium	High		
Total PFAS	*n*	3,913	5,491	2,343	—	—
	MPAH	0.118 ± 0.002 0.100 (0.070, 0.200)	0.253 ± 0.006 0.300 (0.120, 0.400)	0.385 ± 0.006 0.300 (0.300, 0.700)	<0.001	<0.001
	PFDE	0.159 ± 0.002 0.180 (0.100, 0.200)	0.241 ± 0.002 0.200 (0.200, 0.300)	0.701 ± 0.010 0.600 (0.500, 0.900)	<0.001	<0.001
	PFHS	0.781 ± 0.014 0.900 (0.500, 1.400)	2.193 ± 0.029 2.100 (1.300, 3.400)	2.554 ± 0.052 2.500 (1.520, 4.100)	<0.001	<0.001
	PFNA	0.605 ± 0.008 0.656 (0.440, 0.900)	0.956 ± 0.008 1.000 (0.738, 1.312)	2.301 ± 0.031 2.132 (1.640, 3.000)	<0.001	<0.001
	PFOA	1.607 ± 0.020 1.770 (1.200, 2.400)	3.920 ± 0.033 3.900 (2.900, 5.300)	5.324 ± 0.083 5.400 (3.700, 7.700)	<0.001	<0.001
	PFOS	4.293 ± 0.064 4.860 (3.080, 6.890)	15.609 ± 0.157 15.200 (10.500, 22.700)	23.134 ± 0.398 22.600 (14.600, 35.400)	<0.001	<0.001
	PFUA	0.113 ± 0.001 0.100 (0.070, 0.160)	0.158 ± 0.001 0.140 (0.140, 0.200)	0.423 ± 0.008 0.400 (0.270, 0.600)	<0.001	<0.001
PFAS excluding PFOA	*n*	4,160	5,327	2,260	—	—
	MPAH	0.117 ± 0.002 0.100 (0.070, 0.200)	0.234 ± 0.006 0.300 (0.120, 0.400)	0.427 ± 0.006 0.300 (0.300, 0.700)	<0.001	<0.001
	PFDE	0.163 ± 0.002 0.190 (0.100, 0.200)	0.246 ± 0.002 0.200 (0.200, 0.300)	0.709 ± 0.011 0.640 (0.500, 0.900)	<0.001	<0.001
	PFHS	0.835 ± 0.015 0.910 (0.500, 1.500)	2.244 ± 0.030 2.120 (1.370, 3.500)	2.471 ± 0.052 2.400 (1.500, 3.960)	<0.001	<0.001
	PFNA	0.633 ± 0.008 0.700 (0.492, 0.920)	0.965 ± 0.009 1.000 (0.710, 1.340)	2.282 ± 0.032 2.110 (1.600, 3.034)	<0.001	<0.001
	PFOS	4.522 ± 0.065 5.100 (3.200, 7.300)	16.532 ± 0.170 16.200 (11.200, 24.100)	22.319 ± 0.392 21.900 (14.000, 34.300)	<0.001	<0.001
	PFUA	0.112 ± 0.001 0.100 (0.070, 0.150)	0.157 ± 0.002 0.180 (0.140, 0.200)	0.444 ± 0.008 0.400 (0.300, 0.700)	<0.001	<0.001
PFAS excluding PFOS	*n*	4,133	5,180	2,434	—	—
	MPAH	0.117 ± 0.001 0.100 (0.070, 0.200)	0.222 ± 0.005 0.300 (0.1100, 0.300)	0.442 ± 0.006 0.400 (0.300, 0.710)	<0.001	<0.001
	PFDE	0.161 ± 0.002 0.190 (0.100, 0.200)	0.241 ± 0.002 0.200 (0.200, 0.300)	0.668 ± 0.010 0.600 (0.400, 0.900)	<0.001	<0.001
	PFHS	0.807 ± 0.014 0.900 (0.500, 1.400)	2.305 ± 0.031 2.200 (1.400, 3.500)	2.370 ± 0.048 2.300 (1.400, 3.800)	<0.001	0.028
	PFNA	0.611 ± 0.007 0.660 (0.480, 0.902)	0.959 ± 0.008 1.000 (0.700, 1.312)	2.216 ± 0.030 2.050 (1.558, 2.900)	<0.001	<0.001
	PFOA	1.677 ± 0.021 1.850 (1.250, 2.570)	3.994 ± 0.036 4.000 (2.900, 5.500)	5.070 ± 0.078 5.100 (3.500, 7.400)	<0.001	<0.001
	PFUA	0.113 ± 0.001 0.100 (0.070, 0.160)	0.157 ± 0.002 0.140 (0.140, 0.200)	0.413 ± 0.008 0.400 (0.230, 0.600)	<0.001	<0.001

Note: GMs and medians were weight-adjusted using NHANES-specified sampling weights. *p*-Values were calculated using Mann–Whitney U test. Samples with test values below the LOQ were substituted with the value of the LOQ divided by the square root of 2. —, not applicable; GM, geometric mean; LOQ, limit of quantification; MPAH, 2-(*N*-methyl-perfluorooctane sulfonamido) acetic acid; NHANES, National Health and Nutrition Examination Survey; PFAS, per- and polyfluoroalkyl substances; PFDE, perfluorodecanoic acid; PFHS, perfluorohexane sulfonate acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFUA, perfluoroundecanoic acid; SE, standard error.

and perfluoroheptanoic acid (PFHP)], whose detection rates in the population were ∼10% or less, seven PFAS [2-(*N*-methyl-perfluorooctane sulfonamido) acetic acid (MPAH), perfluorodecanoic acid (PFDE), perfluorohexane sulfonate acid (PFHS), perfluorononanoic acid (PFNA), PFOA, PFOS, and perfluoroundecanoic acid (PFUA)] were included in the mixture analysis. A total of 11,747 participants with valid serum concentrations of these seven PFAS were included in the present study. The highest detection rates were observed for PFOS and PFOA, with ∼99% of participants showing serum concentrations higher than the LOQ (Table S3). Concentrations of serum PFOA and PFOS, which ranged from 0.07 to 123 and from 0.07 to 435 ng/mL, respectively, were both skewed distributed with long tails to the right (Figure S1). The medians and quartiles of serum PFOA and PFOS concentrations at baseline were 3.27 (2.00, 5.00) and 11.60 (6.40, 22.40) ng/mL, and the geometric mean (GM) concentrations [mean ± standard error (SE)] were 3.09 ± 0.03 and 10.96 ± 0.12 ng/mL, respectively (Table S3). The distributions of the other five PFAS were also skewed with long tails to the right (Figure S1), and the concentrations and detection rates are provided in Table S3. The largest correlation was observed between serum concentrations of PFUA and PFDE (correlation = 0.830). Serum concentrations of other PFAS showed weak-to-moderate correlations ($0.02 < \text{correlation} \leq 0.50$) (Figure 1).

Confounders for PFAS Mixture/PFOA/PFOS Exposure

A total of 11,747 participants were clustered into three subgroups according to the serum concentrations of seven PFAS (Figure 2A), PFAS excluding PFOA (Figure 2B), and PFAS excluding PFOS (Figure 2C). Concentrations of each PFAS were significantly different among the three subgroups, with the highest serum concentrations in the high-exposure group and the lowest concentrations in the low-exposure group (Table 1). Exposure levels of PFAS mixture/PFOA/PFOS were significantly associated with sex, age, race/ethnicity, smoking status, physical

Table 2. Baseline characteristics [*n* (%)] of participants in the NHANES follow-up study from 1999–2014 to 31 December 2015 ($n = 11{,}747$).

		Total serum PFAS				Serum PFOA concentration				Serum PFOS concentration			
Characteristics	Total ($n = 11{,}747$)	Low ($n = 3{,}913$)	Medium ($n = 5{,}491$)	High ($n = 2{,}343$)	*p*-Value	Tertile 1 <2.4 ng/mL ($n = 3{,}913$)	Tertile 2 2.4–4.3 ng/mL ($n = 3{,}842$)	Tertile 3 ≥4.3 ng/mL ($n = 3{,}992$)	*p*-Value	Tertile 1 <7.9 ng/mL ($n = 3{,}886$)	Tertile 2 7.9–17.1 ng/mL ($n = 3{,}917$)	Tertile 3 ≥17.1 ng/mL ($n = 3{,}944$)	*p*-Value
Deaths	1,251 (7.0)	313 (4.4)	532 (6.4)	406 (13.2)	<0.001	322 (4.6)	418 (7.2)	511 (9.1)	<0.001	199 (2.7)	372 (6.0)	680 (12.2)	<0.001
Heart disease	372 (2.1)	81 (1.0)	184 (2.2)	107 (3.5)	<0.001	100 (1.4)	128 (2.2)	144 (2.6)	0.158	62 (0.7)	129 (2.2)	181 (3.3)	<0.001
Cancer	248 (1.4)	43 (0.4)	135 (1.7)	70 (2.2)	0.002	59 (0.7)	74 (1.2)	115 (2.3)	0.008	39 (0.4)	79 (1.3)	130 (2.5)	<0.001
PFAS excluding PFOA (exposure)													
Low	4,160 (36.2)	—	—	—	—	2,802 (74.1)	1,124 (32.7)	234 (5.9)	<0.001	—	—	—	—
Medium	5,327 (44.0)	—	—	—	—	808 (19.6)	2,113 (52.5)	2,406 (57.9)	<0.001	—	—	—	—
High	2,260 (19.8)	—	—	—	—	303 (6.3)	605 (14.8)	1,352 (36.2)	<0.001	—	—	—	—
PFAS excluding PFOS (exposure)													
Low	4,133 (35.4)	—	—	—	—	—	—	—	—	3,028 (76.6)	979 (23.3)	126 (2.9)	<0.001
Medium	5,180 (42.7)	—	—	—	—	—	—	—	—	666 (18.3)	2,076 (52.9)	2,438 (58.5)	<0.001
High	2,434 (21.9)	—	—	—	—	—	—	—	—	192 (5.1)	862 (23.8)	1,380 (38.5)	<0.001
Sex													
Male	5,681 (48.5)	1,401 (36.4)	2,924 (53.7)	1,356 (56.6)	<0.001	1,391 (34.8)	1,927 (50.1)	2,363 (59.3)	<0.001	1,382 (35.2)	1,918 (50.3)	2,381 (61.3)	<0.001
Female	6,066 (51.5)	2,512 (63.6)	987 (46.3)	2,567 (43.4)	<0.001	2,522 (65.2)	1,915 (49.9)	1,629 (40.7)	<0.001	2,504 (64.8)	1,999 (49.7)	1,563 (38.7)	<0.001
Age (y)[a]	42.5	39.6	43.2	45.7	<0.001	40.3	43.2	43.8	<0.001	39.3	42.6	46.2	<0.001
Race/ethnicity													
Non-Hispanic White	5,274 (69.4)	1,426 (60.8)	2,877 (77.0)	971 (66.5)	<0.001	1,357 (58.3)	1,722 (71.1)	2,195 (77.9)	<0.001	1,527 (63.1)	1,794 (71.8)	1,953 (73.9)	<0.001
Non-Hispanic Black	2,427 (10.8)	730 (11.1)	1,002 (9.1)	695 (14.2)	0.001	852 (13.3)	782 (10.6)	793 (8.8)	0.005	674 (10.0)	761 (10.1)	992 (12.4)	0.148
Mexican American	2,219 (8.5)	895 (13.1)	1,111 (6.9)	213 (4.4)	<0.001	875 (12.7)	776 (8.2)	568 (4.9)	<0.001	821 (12.2)	788 (8.3)	610 (4.6)	<0.001
Other	1,827 (11.3)	862 (14.9)	501 (7.0)	464 (14.9)	<0.001	829 (15.7)	562 (10.1)	436 (8.4)	<0.001	864 (14.7)	574 (9.8)	389 (9.1)	<0.001
Education													
With high school education	5,266 (56.1)	1,790 (56.3)	2,346 (54.8)	1,130 (58.6)	0.225	1,703 (54.5)	1,765 (57.6)	1,798 (56.3)	0.374	1,814 (57.0)	1,804 (57.5)	1,648 (53.4)	0.131
Without high school education	5,606 (40.2)	1,828 (39.3)	2,648 (41.2)	1,130 (39.5)	0.635	1,927 (41.1)	1,780 (38.8)	1,899 (40.6)	0.544	1,772 (38.5)	1,813 (38.8)	2,021 (43.7)	0.029
Missing	875 (3.7)	295 (4.4)	497 (4.0)	83 (1.8)	0.004	283 (4.4)	297 (3.6)	295 (3.1)	0.299	300 (7.5)	300 (7.2)	275 (7.6)	0.939
Income													
≥$20,000	8,443 (80.7)	2,826 (79.7)	3,863 (80.2)	1,754 (83.5)	0.062	2,785 (78.9)	2,718 (80.3)	2,940 (82.7)	0.094	2,811 (79.0)	2,828 (82.0)	2,804 (81.1)	0.218
<$20,000	2,654 (15.3)	874 (16.0)	1,318 (16.0)	462 (12.7)	0.057	926 (17.4)	890 (15.1)	838 (13.8)	0.078	868 (16.7)	892 (14.7)	894 (14.6)	0.339
Missing	650 (3.9)	213 (4.3)	310 (3.8)	127 (3.8)	0.803	202 (3.8)	234 (4.6)	214 (3.5)	0.428	207 (4.3)	197 (3.3)	246 (4.3)	0.417
Smoking status													
Never	5,989 (52.4)	2,211 (57.2)	2,557 (48.3)	1,221 (53.7)	<0.001	2,242 (57.8)	1,881 (51.0)	1,866 (48.9)	<0.001	2,152 (55.9)	1,985 (52.3)	1,852 (48.7)	0.005
Former smoker	2,739 (23.5)	753 (20.0)	1,354 (25.0)	632 (26.0)	0.003	776 (19.7)	938 (25.0)	1,025 (25.6)	0.003	713 (19.2)	935 (24.3)	1,091 (27.5)	<0.001
Current smoker	2,264 (21.0)	749 (20.1)	1,101 (22.9)	414 (18.6)	0.054	699 (19.6)	753 (20.9)	812 (22.4)	0.306	819 (22.2)	715 (19.9)	730 (21.0)	0.451
Missing	755 (3.0)	200 (2.7)	479 (3.8)	76 (1.7)	0.016	196 (2.8)	270 (3.1)	289 (3.0)	0.922	202 (2.7)	282 (3.4)	271 (2.7)	0.563
Alcohol intake (d/wk)													
<1	5,594 (47.4)	1,904 (49.7)	2,675 (48.3)	1,015 (41.4)	<0.001	1,900 (49.1)	1,856 (47.8)	1,838 (45.4)	0.243	1,844 (49.2)	1,868 (47.9)	1,882 (44.6)	0.409
≥1	3,011 (32.2)	863 (27.9)	1,410 (32.6)	738 (38.1)	<0.001	811 (26.1)	992 (33.1)	1,208 (36.6)	<0.001	968 (31.4)	995 (32.6)	1,048 (32.8)	0.769
Missing	3,142 (20.5)	1,146 (22.4)	1,406 (19.0)	590 (20.5)	0.170	1,202 (24.8)	994 (19.0)	946 (18.0)	<0.001	1,074 (19.4)	1,054 (19.5)	1,014 (22.6)	0.237

Table 2. (*Continued.*)

Characteristics	Total ($n = 11,747$)	Total serum PFAS: Low ($n = 3,913$)	Medium ($n = 5,491$)	High ($n = 2,343$)	p-Value	Serum PFOA concentration: Tertile 1 <2.4 ng/mL ($n = 3,913$)	Tertile 2 2.4–4.3 ng/mL ($n = 3,842$)	Tertile 3 ≥4.3 ng/mL ($n = 3,992$)	p-Value	Serum PFOS concentration: Tertile 1 <7.9 ng/mL ($n = 3,886$)	Tertile 2 7.9–17.1 ng/mL ($n = 3,917$)	Tertile 3 ≥17.1 ng/mL ($n = 3,944$)	p-Value
Physical activity (times/month)													
<15	1,789 (15.7)	218 (4.9)	1,234 (22.5)	337 (17.9)	<0.001	297 (6.9)	555 (13.9)	937 (25.0)	<0.001	167 (3.5)	539 (14.4)	1,083 (30.7)	<0.001
≥15	1,115 (9.4)	148 (3.4)	750 (12.9)	217 (11.7)	<0.001	218 (5.3)	356 (8.9)	541 (13.6)	<0.001	114 (2.8)	350 (8.9)	651 (17.5)	<0.001
Missing	8,843 (74.9)	3,547 (91.7)	3,507 (64.6)	1,789 (70.4)	<0.001	3,398 (87.8)	2,931 (77.1)	2,514 (61.4)	<0.001	3,605 (93.7)	3,028 (76.7)	2,210 (51.8)	<0.001
Hypertension													
With	2,088 (14.8)	528 (12.2)	1,075 (16.1)	485 (16.1)	0.018	568 (12.2)	714 (15.4)	806 (16.5)	0.019	3,223 (11.6)	3,085 (14.4)	2,862 (18.8)	<0.001
Without	9,170 (81.2)	3,219 (83.8)	4,207 (80.1)	1,744 (79.2)	0.021	3,150 (83.2)	2,988 (81.0)	3,032 (79.5)	0.103	503 (84.4)	679 (82.0)	906 (76.6)	<0.001
Missing	489 (4.1)	166 (4.0)	209 (3.8)	114 (4.7)	0.572	195 (4.6)	140 (3.6)	154 (3.9)	0.506	160 (4.0)	154 (3.7)	175 (4.6)	0.586
Diabetes													
With	1,282 (8.8)	438 (9.6)	575 (8.3)	269 (8.4)	0.520	501 (10.6)	429 (9.1)	352 (6.7)	0.008	400 (9.1)	434 (8.3)	448 (8.9)	0.805
Without	10,236 (89.5)	3,397 (88.3)	4,822 (90.3)	2,017 (89.7)	0.328	3,339 (87.6)	3,336 (88.9)	3,561 (91.8)	0.070	3,415 (89.1)	3,406 (90.1)	3,415 (89.3)	0.742
Missing	229 (1.7)	78 (2.1)	93 (1.4)	57 (1.9)	0.479	73 (1.8)	77 (1.9)	79 (1.5)	0.775	71 (1.8)	77 (1.6)	81 (1.8)	0.925
Healthy eating index (tertile)													
First	2,332 (18.9)	594 (14.2)	1,179 (20.5)	559 (22.9)	<0.001	694 (17.0)	723 (17.7)	915 (22.0)	0.008	636 (15.6)	764 (19.4)	932 (22.7)	<0.001
Second	2,350 (18.9)	846 (20.5)	1,081 (18.6)	423 (17.2)	0.531	834 (20.4)	779 (19.5)	737 (17.7)	0.296	898 (22.2)	776 (18.7)	676 (16.1)	0.002
Third	2,344 (18.9)	930 (22.6)	1,094 (19.0)	320 (12.7)	<0.001	835 (20.1)	869 (21.5)	640 (15.0)	<0.001	842 (20.7)	795 (17.8)	707 (17.1)	0.089
Missing	4,721 (43.2)	1,543 (42.7)	2,137 (41.9)	1,041 (47.2)	0.036	1,550 (42.5)	1,471 (41.3)	1,700 (45.3)	0.179	1,510 (41.5)	1,582 (44.1)	1,629 (44.1)	0.399
BMI (kg/m^2)													
Normal (<25.0)	3,672 (31.9)	1,166 (30.1)	1,746 (32.3)	760 (33.9)	0.188	1,224 (31.4)	1,221 (32.7)	1,227 (31.6)	0.798	1,228 (32.5)	1,236 (32.7)	1,208 (30.2)	0.411
Overweight (25.0–29.9)	3,867 (33.4)	1,191 (30.0)	1,855 (34.7)	821 (36.1)	0.011	1,190 (30.9)	1,274 (33.3)	1,403 (35.5)	0.090	1,175 (30.8)	1,310 (34.4)	1,382 (35.2)	0.080
Obese (≥30.0)	4,039 (33.5)	1,482 (38.3)	1,819 (32.0)	738 (29.3)	<0.001	1,411 (35.6)	1,303 (33.0)	1,325 (32.2)	0.243	1,416 (35.1)	1,319 (31.9)	1,304 (33.6)	0.317
Missing	169 (1.2)	74 (1.7)	71 (1.0)	24 (0.7)	0.095	88 (2.0)	44 (0.9)	37 (0.7)	0.016	67 (1.6)	52 (1.0)	50 (0.9)	0.288
Ccr (mL/min)													
≥70	9,826 (87.2)	3,431 (89.9)	4,505 (86.0)	1,890 (85.5)	0.006	3,319 (87.9)	3,222 (87.3)	3,285 (86.5)	0.642	3,425 (90.0)	3,282 (87.7)	3,119 (83.5)	<0.001
50–70	1,101 (7.6)	238 (5.1)	582 (8.6)	281 (9.6)	<0.001	295 (5.9)	372 (8.3)	434 (8.6)	0.044	237 (5.4)	377 (7.6)	487 (10.2)	<0.001
30–49.9	543 (3.3)	122 (2.5)	290 (3.8)	131 (3.7)	0.197	151 (3.1)	183 (3.2)	209 (3.7)	0.726	111 (2.3)	177 (3.2)	255 (4.7)	0.011
<30	126 (0.7)	60 (1.0)	47 (0.6)	19 (0.4)	0.244	69 (1.2)	28 (0.5)	29 (0.4)	0.065	55 (0.9)	33 (0.6)	38 (0.6)	0.649
Missing	151 (1.2)	62 (1.5)	67 (1.1)	22 (0.7)	0.230	79 (1.9)	37 (0.8)	35 (0.8)	0.030	58 (1.4)	48 (1.0)	45 (1.1)	0.687
Total cholesterol (mg/dL)													
Concentration[a,b]	11,744 (191.2)	3,912 (186.9)	5,489 (192.3)	2,343 (195.9)	<0.001	3,912 (185.9)	3,842 (191.4)	3,990 (195.7)	<0.001	3,886 (187.3)	3,916 (189.7)	3,942 (197.3)	<0.001
Missing	3 (—)	1 (—)	2 (—)	0 (—)	—	1 (—)	0 (—)	2 (—)	—	0 (—)	1 (—)	2 (—)	—
Serum cotinine (ng/dL)													
Concentration[a,b]	11,705 (0.37)	3,907 (0.26)	5,457 (0.50)	2,341 (0.32)	<0.001	3,901 (0.26)	3,835 (0.35)	3,969 (0.53)	<0.001	3,882 (0.32)	3,912 (0.34)	3,911 (0.48)	<0.001
Missing	42 (—)	6 (—)	34 (—)	2 (—)	—	12 (—)	7 (—)	23 (—)	—	4 (—)	5 (—)	33 (—)	—

Note: Percentages, means, and cut points were weight-adjusted using NHANES-specified sampling weights. For categorical variables, p-values were calculated using chi-square test, and for continuous variables, p-values were calculated using one-way ANOVA (normal distribution) or Kruskal–Wallis H test (non-normal distribution). Samples with test values below the LOQ were substituted with the value of the LOQ divided by the square root of 2. —, not applicable; ANOVA, analysis of variance; BMI, body mass index; Ccr, creatinine clearance rate; GM, geometric mean; LOQ, limit of quantification; NHANES, National Health and Nutrition Examination Survey; PFAS, per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid.

[a]Age, total cholesterol, and cotinine were treated as continuous variables.

[b]Non-normal distribution data presented as GMs.

Table 3. HRs (95% CIs) for PFAS mixture/PFOA/PFOS exposure and mortality risk in the NHANES follow-up study from 1999–2014 to 31 December 2015 ($n = 11,747$).

Categories	All-cause mortality			Heart disease mortality			Cancer mortality		
	Deaths/observations (n/N)	Unadjusted HR	Adjusted HR	Deaths/observations (n/N)	Unadjusted HR	Adjusted HR	Deaths/observations (n/N)	Unadjusted HR	Adjusted HR
Total PFAS (exposure)[a]									
Low	313/3,913	Ref	Ref	81/3,913	Ref	Ref	43/3,913	Ref	Ref
Medium	532/5,491	1.15 (0.90, 1.45)	1.02 (0.78, 1.33)	184/5,491	1.38 (0.98, 1.87)	1.14 (0.76, 1.74)	135/5,491	1.58 (1.05, 2.46)	1.31 (0.83, 2.16)
High	406/2,343	1.62 (1.33, 1.97)	1.38 (1.07, 1.80)	107/2,343	1.81 (1.20, 2.62)	1.58 (1.05, 2.51)	70/2,343	2.05 (1.27, 3.19)	1.70 (1.08, 2.84)
PFOA [tertile (ng/mL)][b]									
First (<2.4)	322/3,913	Ref	Ref	100/3,913	Ref	Ref	59/3,913	Ref	Ref
Second (2.4–4.3)	418/3,842	1.29 (1.06, 1.58)	1.10 (0.86, 1.41)	128/3,842	1.28 (0.92, 1.79)	1.13 (0.78, 1.67)	74/3,842	1.10 (0.70, 1.70)	0.99 (0.60, 1.62)
Third (≥4.3)	511/3,992	1.34 (1.11, 1.61)	1.22 (0.93, 1.58)	144/3,992	1.30 (0.96, 1.83)	1.15 (0.73, 1.73)	115/3,992	1.23 (0.83, 1.83)	1.06 (0.68, 1.71)
PFOS [tertile (ng/mL)][c]									
First (<7.9)	199/3,886	Ref	Ref	62/3,886	Ref	Ref	39/3,886	Ref	Ref
Second (7.9–17.1)	372/3,917	1.36 (1.08, 1.70)	1.13 (0.86, 1.44)	129/3,917	1.40 (0.96, 2.05)	1.19 (0.78, 1.80)	79/3,917	1.45 (0.95, 2.25)	1.26 (0.75, 2.06)
Third (≥17.1)	680/3,944	1.83 (1.50, 2.31)	1.57 (1.22, 2.07)	181/3,944	1.88 (1.34, 2.75)	1.65 (1.09, 2.57)	130/3,944	2.13 (1.41, 3.27)	1.75 (1.10, 2.83)

Note: HRs were estimated using Cox proportional hazards models and were weight adjusted using NHANES-specified sampling weights. Missing data on covariates were processed using the multiple imputation algorithm. Samples with test values below the LOQ were substituted with the value of the LOQ divided by the square root of 2. BMI, body mass index; Ccr, creatinine clearance rate; CI, confidence interval; HR, hazard ratio; LOQ, limit of quantification; NHANES, National Health and Nutrition Examination Survey; PFAS, per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; Ref, reference.

[a]HRs were adjusted for sex, age, race/ethnicity, smoking status, alcohol intake, physical activity, hypertension, healthy eating index, BMI, Ccr, serum total cholesterol, and serum cotinine.

[b]HRs were adjusted for PFAS excluding PFOA (categorized into three groups based on *k*-means algorithm), sex, age, race/ethnicity, smoking status, alcohol intake, physical activities, hypertension, diabetes, healthy eating index, Ccr, serum total cholesterol, and serum cotinine.

[c]HRs were adjusted for PFAS excluding PFOS (categorized into three groups based on *k*-means algorithm), sex, age, race/ethnicity, education, smoking status, physical activity, hypertension, healthy eating index, Ccr, serum total cholesterol, and serum cotinine.

activity, hypertension, dietary habits, renal function, and serum total cholesterol and serum cotinine concentrations (Table 2). Other confounders included alcohol consumption and BMI for PFAS mixture exposure; alcohol consumption, diabetes, and PFAS mixture (excluding PFOA) for PFOA exposure; education and PFAS mixture (excluding PFOS) for PFOS exposure (Table 2). Confounders for PFAS mixture (excluding PFOA) exposure included age, sex, race/ethnicity, smoking status, alcohol consumption, physical activity, hypertension, BMI, Ccr, and serum total cholesterol, serum cotinine, and serum PFOA concentrations. Confounders for PFAS mixture (excluding PFOS) included age, sex, race/ethnicity, family income, smoking status, alcohol consumption, physical activity, hypertension, BMI, Ccr, and serum total cholesterol, serum cotinine, and serum PFOS concentrations (Table S4).

Association between PFAS Exposure and Risk of Mortality

During the median follow-up of 81 months (interquartile range: 46–112 months), 1,251 participants died. Of these deaths, 29.7% (372) were from heart disease and 19.8% (248) from cancer. After adjusting for confounders, higher levels of PFAS mixture were significantly associated with higher risk of all-cause mortality (high- vs. low-exposure group HR = 1.38; 95% CI: 1.07, 1.80), heart disease mortality (high- vs. low-exposure group HR = 1.58; 95% CI: 1.05, 2.51), and cancer mortality (high- vs. low-exposure group HR = 1.70; 95% CI: 1.08, 2.84) (Table 3). Further analysis on single PFAS by restricted cubic splines showed that serum PFOS concentrations were positively correlated with all-cause, heart disease, and cancer mortality (Figure 3A–C), whereas serum PFOA concentrations had no significant association with mortality (Figure 3D–F). Positive associations between PFOS exposure and all-cause mortality (third tertile vs. first tertile HR = 1.57; 95% CI: 1.22, 2.07), heart disease mortality (third tertile vs. first tertile HR = 1.65; 95% CI: 1.09, 2.57), and cancer mortality (third tertile vs. first tertile HR = 1.75; 95% CI: 1.10, 2.83) were also observed in the adjusted categorical models based on the tertiles of serum PFOS concentrations (Table 3 and Figure 4). There was no significant association between PFOA and mortality risk in the categorical analysis (third tertile vs. first tertile, all-cause mortality HR = 1.22; 95% CI: 0.93, 1.58; heart disease mortality HR = 1.15; 95% CI: 0.73, 1.73; cancer mortality HR = 1.06; 95% CI: 0.68, 1.71) (Table 3). Associations between other key characteristics and mortality are also shown in Figure 4. Notably, we found that the positive association between PFAS mixture exposure and mortality persisted after excluding PFOA (high- vs. low-exposure group, all-cause mortality HR = 1.40; 95% CI: 1.08, 1.86; heart disease mortality HR = 1.54; 95% CI: 1.03, 2.32; cancer mortality HR = 1.68; 95% CI: 1.06, 2.89), whereas the positive correlation between PFAS mixture and mortality no longer existed after excluding PFOS (high- vs. low-exposure group, all-cause mortality HR = 0.86; 95% CI: 0.65, 1.13; heart disease mortality HR = 1.08; 95% CI: 0.69, 1.80; cancer mortality HR = 0.93; 95% CI: 0.55, 1.57) (Table 4).

Number of Deaths Potentially Attributed to PFOS Exposure

We next evaluated the population attributable fractions to estimate the proportional reduction in all-cause, heart disease, and cancer mortality that would occur if serum PFOS concentrations declined from ≥17.1 to <7.9 ng/mL in U.S. adults. The adjusted population attributable fraction for all-cause mortality was 15.4% (95% CI: 7.1, 23.7), equivalent to 382,000 (95% CI: 176,000, 588,000) deaths/y (Table 5). Adjusted population attributable fractions were 16.9% (95% CI: 3.1, 29.2) for heart disease mortality and 18.7% (95% CI: 3.5, 31.1) for cancer mortality, equivalent

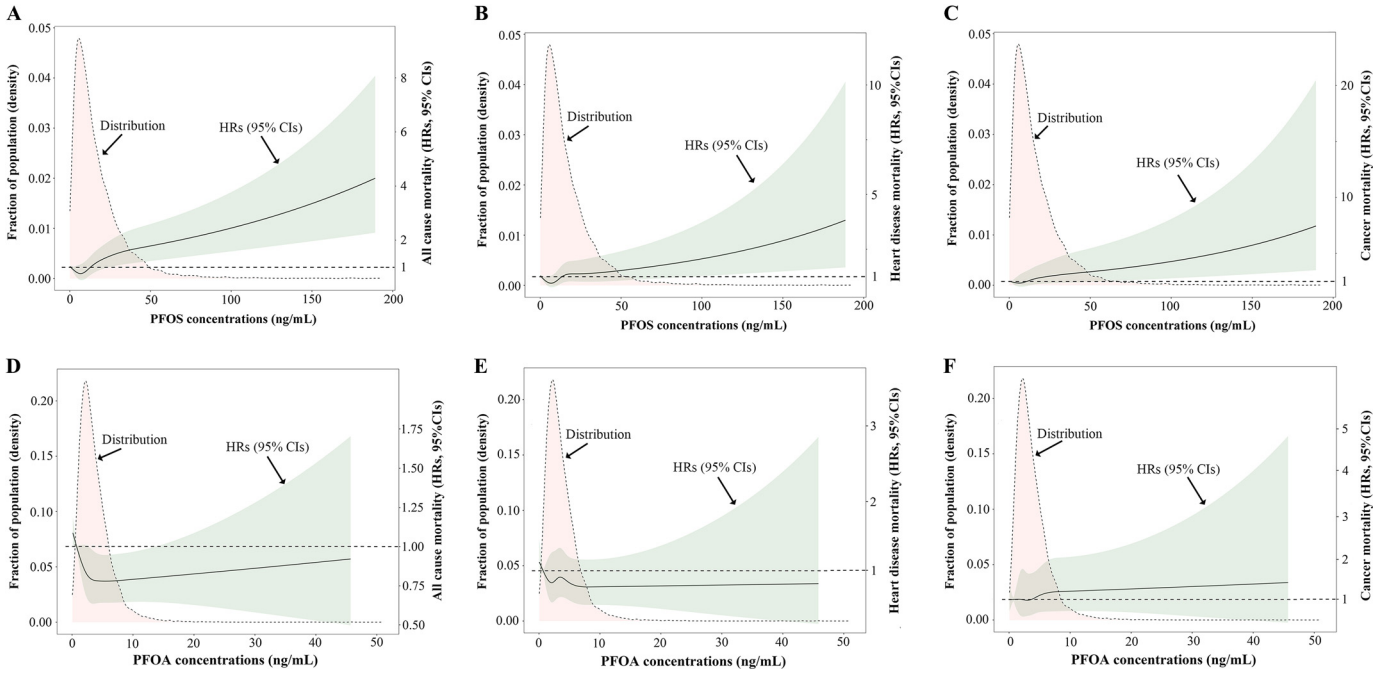

Figure 3. Distributions of serum PFOA/PFOS concentrations and dose-response curves of serum PFOA/PFOS concentrations and mortality in the NHANES follow-up study from 1999–2014 to 31 December 2015 ($n = 11,747$). Distributions of serum PFOS concentrations and adjusted HRs with 95% CIs for (A) all-cause mortality, (B) heart disease mortality, and (C) cancer mortality. Distributions of serum PFOA concentrations and adjusted HRs with 95% CIs for (D) all-cause mortality, (E) heart disease mortality, and (F) cancer mortality. HRs were estimated using Cox proportional hazards models and were weight adjusted using NHANES-specified sampling weights. HRs for PFOS exposure were further adjusted for PFAS excluding PFOS (categorized into three groups based on *k*-means algorithm), sex, age, race/ethnicity, education, smoking status, physical activity, hypertension, healthy eating index, Ccr, serum total cholesterol, and serum cotinine. HRs for PFOA exposure were further adjusted for PFAS excluding PFOA (categorized into three groups based on *k*-means algorithm), sex, age, race/ethnicity, smoking status, alcohol intake, physical activities, hypertension, diabetes, healthy eating index, Ccr, serum total cholesterol, and serum cotinine. Missing data on covariates were processed using multiple imputation algorithm. Samples with test values below the LOQ were substituted with the value of the LOQ divided by the square root of 2. Note: Ccr, creatinine clearance rate; CI, confidence interval; HR, hazard ratio; LOQ, limit of quantification; NHANES, National Health and Nutrition Examination Survey; PFAS, per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid.

Factor	All cause mortality			Heart disease mortality			Cancer mortality		
	Number of deaths/Number of observations	Forest plots	HRs (95% CIs)	Number of deaths/Number of observations	Forest plots	HRs (95% CIs)	Number of deaths/Number of observations	Forest plots	HRs (95% CIs)
PFOS									
Tertile 1 (Ref)	199/3886			62/3886			39/3886		
Tertile 2	372/3917		1.13 (0.86, 1.44)	129/3917		1.19 (0.78, 1.80)	79/3917		1.26 (0.75, 2.06)
Tertile 3	680/3944		1.57 (1.22, 2.07)	181/3944		1.65 (1.09, 2.57)	130/3944		1.75 (1.10, 2.83)
Sex									
Female (Ref)	524/6066			152/6066			99/6066		
Male	727/5681		1.71 (1.46, 2.00)	220/5681		2.01 (1.55, 2.62)	149/5681		1.46 (0.99, 2.12)
Age (Continous)	-		1.08 (1.07, 1.09)	-		1.08 (1.07, 1.10)	-		1.08 (1.07, 1.10)
Race/ethnicity									
Non-Hispanic white (Ref)	734/5274			219/5274			144/5274		
Mexican-American	205/2219		1.16 (0.91, 1.49)	52/2219		1.06 (0.67, 1.67)	34/2219		1.14 (0.62, 1.98)
Non-Hispanic black	222/2427		1.31 (1.08, 1.57)	68/2427		1.30 (0.93, 1.82)	50/2427		1.24 (0.75, 2.10)
Other	90/1827		1.15 (0.95, 1.39)	33/1827		1.25 (0.91, 1.73)	20/1827		1.35 (0.83, 2.15)
Education									
Without high school education (Ref)	845/5940			252/5940			153/5940		
With high school education	406/5807		0.75 (0.64, 0.88)	120/5807		0.73 (0.55, 0.95)	95/5807		0.98 (0.71, 1.37)
Smoking									
Never (Ref)	527/6414			155/6414			81/6414		
Current smoker	511/2861		1.24 (1.05, 1.47)	152/2861		1.19 (0.89, 1.59)	115/2861		1.79 (1.19, 2.68)
Former smoker	213/2472		1.93 (1.54, 2.43)	65/2472		1.65 (1.10, 2.49)	52/2472		2.74 (1.67, 4.49)
Physical activity									
0-14 times/month (Ref)	777/7259			238/7259			161/7259		
≥ 15 times/month	474/4488		0.93 (0.79, 1.09)	134/4488		0.89 (0.67, 1.19)	87/4488		0.94 (0.66, 1.34)
Hypertension									
Without hypertension (Ref)	749/9566			210/9566			171/9566		
With hypertension	502/2181		1.14 (0.98, 1.33)	162/2181		1.41 (1.08, 1.85)	77/2181		0.81 (0.58, 1.13)
Healthy eating habits									
Tertile 1 (Ref)	627/3943			168/3943			107/3943		
Tertile 2	387/3876		0.84 (0.67, 1.04)	147/3876		0.85 (0.58, 1.18)	99/3876		0.95 (0.60, 1.54)
Tertile 3	237/3928		0.77 (0.62, 0.95)	57/3928		0.63 (0.41, 0.90)	42/3928		0.75 (0.46, 1.20)
Ccr (mL/min)									
≥ 70 (Ref)	627/9948			168/9948			154/9948		
50 to 70	289/1119		1.28 (1.03, 1.58)	80/1119		1.11 (0.77, 1.61)	53/1119		1.18 (0.70, 1.99)
30 to 49.9	261/554		1.97 (1.55, 2.50)	94/554		2.29 (1.53, 3.43)	34/554		0.95 (0.53, 1.68)
< 30	74/126		3.51 (2.53, 4.88)	30/126		4.25 (2.42, 7.47)	7/126		1.79 (0.68, 4.71)

Figure 4. Adjusted HRs with 95% CIs for all-cause, heart disease, and cancer mortality in the NHANES follow-up study from 1999–2014 to 31 December 2015 ($n = 11,747$). Adjusted HRs are shown as green squares, orange dots, and cyan diamonds and 95% CIs as horizontal lines for all-cause, heart disease, and cancer mortality, respectively. Age variables were treated as continuous values. HRs were estimated using Cox proportional hazards models and were weight adjusted using NHANES-specified sampling weights. Other adjusted variables include PFAS excluding PFOS (categorized into three groups based on *k*-means algorithm), sex, age, race/ethnicity, high school education, smoking status, hypertension, healthy eating index, serum total cholesterol, and serum cotinine. Missing data on covariates were processed using multiple imputation algorithm. Samples with test values below the LOQ were substituted with the value of the LOQ divided by the square root of 2. Note: CI, confidence interval; Ccr, creatinine clearance rate; HR, hazard ratio; LOQ, limit of quantification; NHANES, National Health and Nutrition Examination Survey; PFAS, per- and polyfluoroalkyl substances; PFOS, perfluorooctane sulfonic acid; Ref, reference.

Table 4. HRs (95% CIs) for PFAS (excluding PFOA) Mixture/PFAS (excluding PFOS) mixture exposure and mortality risk in the NHANES follow-up study from 1999–2014 to 31 December 2015 ($n = 11,747$).

	All-cause mortality			Heart disease mortality			Cancer mortality		
Categories	Deaths/observations (n/N)	Unadjusted HR	Adjusted HR	Deaths/observations (n/N)	Unadjusted HR	Adjusted HR	Deaths/observations (n/N)	Unadjusted HR	Adjusted HR
PFAS excluding PFOA (exposure)[a]									
Low	216/4,160	Ref	Ref	77/4,160	Ref	Ref	56/4,160	Ref	Ref
Medium	717/5,327	1.26 (1.08, 1.51)	1.08 (0.84, 1.39)	203/5,327	1.60 (1.15, 2.18)	1.30 (0.88, 1.92)	118/5,327	1.21 (0.77, 1.93)	1.08 (0.68, 1.83)
High	318/2,260	1.63 (1.30, 1.99)	1.40 (1.08, 1.86)	92/2,260	1.73 (1.25, 2.33)	1.54 (1.03, 2.32)	74/2,260	1.93 (1.27, 3.05)	1.68 (1.06, 2.89)
PFAS excluding PFOS (exposure)[b]									
Low	375/4,133	Ref	Ref	104/4,133	Ref	Ref	75/4,133	Ref	Ref
Medium	653/5,180	1.34 (1.09, 1.64)	1.11 (0.90, 1.44)	191/5,180	1.40 (1.05, 1.97)	1.16 (0.79, 1.69)	116/5,180	1.01 (0.65, 1.60)	0.86 (0.53, 1.36)
High	223/2,434	1.04 (0.88, 1.24)	0.86 (0.65, 1.13)	77/2,434	1.25 (0.90, 1.76)	1.08 (0.69, 1.80)	57/2,434	1.04 (0.70, 1.63)	0.93 (0.55, 1.57)

Note: HRs were estimated using Cox proportional hazards models and were weight adjusted using NHANES-specified sampling weights. Missing data on covariates were processed using multiple imputation algorithm. Samples with test values below the LOQ were substituted with the value of the LOQ divided by the square root of 2. BMI, body mass index; Ccr, creatinine clearance rate; CI, confidence interval; HR, hazard ratio; LOQ, limit of quantification; NHANES, National Health and Nutrition Examination Survey; PFAS, per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; Ref, reference.

[a]HRs were adjusted for sex, age, race/ethnicity, smoking status, alcohol intake, physical activity, hypertension, BMI, Ccr, serum total cholesterol, serum cotinine, and serum PFOA concentrations (categorized into three groups based on tertiles).

[b]HRs were adjusted for sex, age, race/ethnicity, family income, smoking status, alcohol intake, physical activity, hypertension, BMI, Ccr, serum total cholesterol, serum cotinine, and serum PFOS concentrations (categorized into three groups based on tertiles).

to 109,000 (95% CI: 20,000, 188,000) heart disease deaths and 106,000 (95% CI: 20,000, 176,000) cancer deaths annually from 1999 to 2015 (Table 5).

From 2015 to 2018, with the decline of serum PFOS concentrations among U.S adults, the proportion of the population with serum PFOS concentrations at ≥ 17.1 ng/mL decreased to 4.6%, whereas the proportion of the population with serum PFOS concentrations <7.9 ng/mL increased to 75.0%. Based on the proportion of each category, the estimated population attributable fractions were 2.5% (95% CI: 1.0, 4.3) for all-cause mortality, 2.8% (95% CI: 0.4, 5.8) for heart disease mortality, and 3.2% (95% CI: 0.5, 6.5) for cancer mortality, equivalent to 69,000 (95% CI: 28,000, 119,000) all-cause deaths, 18,000 (95% CI: 3,000, 37,000) heart disease deaths, and 19,000 (95% CI: 3,000, 39,000) cancer deaths annually from 2015 to 2018 (Table 6).

Sensitivity Analysis and Examination of Effect Modification

No appreciable attenuation was observed from the results of the primary analysis on PFOS when we made several adjustments on the evaluation method of covariates, such as other six PFAS exposures, age, blood pressures, Healthy Eating Index, and smoking status, nor in the analysis excluding subjects who died within a year of the blood draw (Table S5).

Results of the examination of effect modification are provided in Table 7 and Tables S6 and S7. Compared with male participants, female participants had significantly higher risk for all-cause mortality [HR = 1.98 (95% CI: 1.37, 2.86) vs. 1.23 (95% CI: 0.85, 1.78); $p < 0.001$ for interaction] (Table 7). Participants without diabetes showed significantly higher risk than participants with diabetes for all-cause mortality [HR = 1.76 (95% CI: 1.30, 2.39) vs. 1.13 (95% CI: 0.67, 1.88); $p = 0.011$ for interaction] (Table 7).

Discussion

Results of our study suggest that serum PFAS were associated with increased risks of all-cause, heart disease, and cancer mortality in U.S. adults. In our study, PFOS contributed a large part to the strength of the PFAS–mortality association. Assuming a causal relationship, PFOS exposure was associated with ~382,000 deaths in U.S. adults annually from 1999 to 2015, and the number decreased to 69,000 annually from 2015 to 2018.

As persistent contaminants, PFAS bioaccumulate via food chains and have a long half-life.[34] In contrast to most persistent organic pollutants, which accumulate in fatty tissues,[35] most PFAS bind to serum proteins, resulting in the highest levels of PFAS in highly perfused tissues, such as the liver and kidneys, rather than lipid tissues.[34] The mechanisms mediating PFAS action are largely unknown. Proliferator-activated receptor alpha (PPARα), a crucial factor regulating lipid metabolism and inflammation,[36] is a potential target of PFAS. However, despite results from laboratory studies that have reported the transcriptional activation of animal and human PPARα-related genes in the liver by PFAS exposure at concentrations >10 μM, those concentrations are several magnitudes above the average serum concentrations in the Western population, suggesting that PPARα may play only a minor role in potential PFAS-mediated adverse effects at environment-relevant doses.[37]

In humans, the clearance half-life is in the range of 2–3 y for PFOA, and 3–4 y for PFOS.[14,38] The long clearance times, together with the relatively high-exposure level peculiarity, accounts for the highest detectable rates and concentrations of PFOS and PFOA in human blood.[9] With growing evidence supporting the links between single PFAS (especially PFOA and PFOS) and human health outcomes, including cancer,[3] dyslipidemia,[14,39]

Table 5. Population attributable fractions [% (95% CI)] of PFOS for all causes, heart disease, and cancer mortality in the NHANES follow-up study from 1999–2014 to 31 December 2015 ($n = 11,747$).

	Deaths/observations (n/N)		Serum PFOS concentrations decline from ≥17.1 to <7.9 ng/mL (tertile 3 to tertile 1)			
Cause of death	<7.9 ng/mL (tertile 1)	≥17.1 ng/mL (tertile 3)	Adjusted HR (95% CI)	Attributable fraction [% (95% CI)]	Average number of deaths (1999–2015) (n)	Avoidable deaths [n (95% CI)]
All causes	199/3,886	680/3,944	1.57 (1.22, 2.07)	15.4 (7.1, 23.7)	2,480,636	382,000 (176,000, 588,000)
Heart disease	62/3,886	181/3,944	1.65 (1.09, 2.57)	16.9 (3.1, 29.2)	643,525	109,000 (20,000, 188,000)
Cancer	39/3,886	130/3,944	1.75 (1.10, 2.83)	18.7 (3.5, 31.1)	567,441	106,000 (20,000, 176,000)

Note: HRs were estimated using Cox proportional hazards models and were weight adjusted using NHANES-specified sampling weights. Other adjusted variables include PFAS excluding PFOS (categorized into three groups based on k-means algorithm), sex, age, race/ethnicity, education, smoking status, physical activity, hypertension, healthy eating index, Ccr, serum total cholesterol, and serum cotinine. Missing data on covariates were processed using multiple imputation algorithm. Samples with test values below the LOQ were substituted with the value of the LOQ divided by the square root of 2. Ccr, creatinine clearance rate; CI, confidence interval; HR, hazard ratio; LOQ, limit of quantification; NHANES, National Health and Nutrition Examination Survey; PFAS, per- and polyfluoroalkyl substances; PFOS, perfluorooctane sulfonic acid.

immune dysfunction,[14] and cardiovascular disease,[40] further research taking into consideration the coexposure of multiple PFAS may provide more reliable evidence to reflect the health impact of PFAS mixtures. Most populations are exposed to the multiple-PFAS mixture. A previous study reported a significantly higher risk for all-cause mortality among people from PFAS-contaminated municipalities in comparison with uncontaminated ones with similar socioeconomic status and smoking habits.[41] Increased risk of mortality was also observed in factory workers producing PFAS compared with metalworking factory workers.[42]

Our study used an unsupervised clustering model and found positive associations between PFAS mixtures and all-cause, heart disease, and cancer mortality. Although no significant association was observed for PFOA, we did find positive associations between PFOS exposure and all-cause, heart disease, and cancer mortality after adjusting for serum concentrations of other PFAS, and there was no significant association between PFAS mixture exposure and mortality after excluding PFOS. These results suggest that PFOS, not PFOA, probably contributes to the strength of the PFAS–mortality association observed in this study. Notably, serum PFOS concentrations in this study were not as high as reported in previous research among people who were exposed to PFAS-contaminated water,[43,44] indicating that people exposed to PFOS at levels lower than those in the contaminated regions may be at risk. Although the mechanism by which PFOS increases cancer mortality is unknown, it was reported to induce/enhance malignant phenotypes in human cells.[45,46] Basic research regarding PFOS and heart disease is more limited than PFOS and cancer. A recent animal study revealed that PFOS induced inflammatory infiltration in rat heart tissues and increased expression levels of myocardial injury markers.[47] However, whether the observed association between PFOS and heart disease mortality is mediated by the direct cardiotoxicity of PFOS or is a consequence of its toxicity to other organs/tissues remains to be explored.

The different outcomes regarding PFOA and PFOS we observed in this study may be explained from two aspects. First is the different toxicological mechanism between PFOA and PFOS. Previous studies have proved that PFOA and PFOS have different actions *in vivo* and induce different phenotypes.[48–51] Besides, the different exposure levels between PFOA and PFOS may also result in the different outcomes (serum PFOS concentrations were significantly higher than PFOA in this study: 10.96 ± 0.12 vs. 3.09 ± 0.03 ng/mL).

Our study also found that the association between PFOS exposure and all-cause mortality was stronger among women. As a chemical that has endocrine disruptor potential,[52] the actions of PFOS *in vivo* are likely to be affected by the types and levels of hormones. Recent studies revealed that PFOS could disturb androgen/estrogen homeostasis in women and interfere with the function of estrogen,[53–55] and that may lead to the increased risk of estrogen-related diseases, such as breast and ovarian cancer,[56,57] resulting in the increased risk of mortality observed in women. We also observed that the PFOS–mortality association was stronger among people without diabetes when compared with people with diabetes; further interventional/experimental studies are needed to identify a potential mechanism for this observation.

Limitations of this study include, first, the potential for residual or unmeasured confounding. Despite adjustment for an extended range of covariates and PFAS mixture, residual confounding with, for example, dietary fiber intake, which was poorly measured in our study but was reported to have a negative association with serum PFAS levels,[58] cannot be entirely ruled out. Other uncommon PFAS and environmental chemicals could also lead to confounding in the observed association. Second, as a volunteer-based cohort, participants in the NHANES were more often people with health-conscious behaviors and higher educational levels than the general U.S. population.[59] Although sample estimates were weighted to reflect the demographic composition at the given time, populations

Table 6. Population attributable fractions [% (95% CI)] of PFOS for all causes, heart disease, and cancer mortality in the NHANES participants from 2015 to 2018 ($n = 3,922$).

	Serum PFOS concentrations decline from ≥17.1 to <7.9 ng/mL			
Cause of death	Adjusted HR (95% CI)[a]	Attributable fraction [% (95% CI)]	Average number of deaths (2015–2018) (n)	Avoidable deaths [n (95% CI)]
All causes	1.57 (1.22, 2.07)	2.5 (1.0, 4.3)	2,777,397	69,000 (28,000, 119,000)
Heart disease	1.65 (1.09, 2.57)	2.8 (0.4, 5.8)	642,985	18,000 (3,000, 37,000)
Cancer	1.75 (1.10, 2.83)	3.2 (0.5, 6.5)	598,087	19,000 (3,000, 39,000)

Note: Ccr, creatinine clearance rate; CIs, confidence intervals; HR, hazard ratio; LOQ, limit of quantification; NHANES, National Health and Nutrition Examination Survey; PFAS, per- and polyfluoroalkyl substances; PFOS, perfluorooctane sulfonic acid.
[a]HRs were derived from the follow-up data from NHANES participants from 1999–2014 to 2015.
HRs were estimated using Cox proportional hazards models and were weight adjusted using NHANES-specified sampling weights. Other adjusted variables include PFAS excluding PFOS (categorized into three groups based on k-means algorithm), sex, age, race/ethnicity, education, smoking status, physical activity, hypertension, healthy eating index, Ccr, serum total cholesterol, and serum cotinine. Missing data on covariates were processed using multiple imputation algorithm. Samples with test values below the LOQ were substituted with the value of the LOQ divided by the square root of 2.

Table 7. Potential effect modification of the key characteristics on the relation between PFOS exposure and all-cause mortality in the NHANES follow-up study from 1999–2014 to 31 December 2015 ($n = 11{,}747$).

Characteristics	Deaths/observations (n/N)		Adjusted HR (95% CI)	p for interaction[a]
	<7.9 ng/mL (tertile 1)	≥17.1 ng/mL (tertile 3)	≥17.1 vs. <7.9 ng/mL (tertile 3 vs. tertile 1)	
Age (y)				0.064
<50	47/2,606	45/1,702	1.03 (0.46, 2.23)	—
≥50	152/1,280	635/2,242	1.35 (1.02, 1.78)	—
Sex				<0.001
Male	102/1,382	418/2,381	1.23 (0.85, 1.78)	—
Female	97/2,504	262/1,563	1.98 (1.37, 2.86)	—
Race/ethnicity				0.556
Non-Hispanic White	112/1,527	419/1,953	1.72 (1.23, 2.40)	—
Non-Hispanic Black	32/674	129/992	2.33 (1.38, 3.91)	—
Mexican American	35/821	94/610	2.16 (1.07, 4.36)	—
Other	20/864	38/389	1.59 (0.69, 3.65)	—
Smoking status				0.127
Never smoke	83/2,259	278/2,012	1.99 (1.34, 2.95)	—
Past or current smoker	116/1,627	402/1,932	1.67 (1.18, 2.36)	—
Hypertension				0.299
No	135/3,356	387/2,997	1.57 (1.11, 2.23)	—
Yes	64/530	293/947	1.59 (1.08, 2.36)	—
Diabetes				0.011
No	139/3,477	530/3,488	1.76 (1.30, 2.39)	—
Yes	60/409	150/456	1.13 (0.67, 1.88)	—
Obesity				0.981
No	128/2,446	461/2,620	1.86 (1.33, 2.60)	—
Yes	71/1,440	219/1,324	1.78 (1.15, 2.73)	—
Renal function [Ccr (mL/min)]				0.291
Normal (≥70)	99/3,472	335/3,157	1.55 (0.89, 2.67)	—
Impaired (<70)	100/414	345/787	0.72 (0.34, 1.56)	—

Note: HRs were estimated using Cox proportional hazards models and were weight adjusted using NHANES-specified sampling weights. Other adjusted variables include PFAS excluding PFOS (categorized into three groups based on *k*-means algorithm), sex, age, race/ethnicity, education, smoking status, physical activity, hypertension, healthy eating index, Ccr, serum total cholesterol, and serum cotinine. Missing data on covariates were processed using multiple imputation algorithm. Samples with test values below the LOQ were substituted with the value of the LOQ divided by the square root of 2. —, not applicable; Ccr, creatinine clearance rate; CI, confidence interval; HR, hazard ratio; LOQ, limit of quantification; NHANES, National Health and Nutrition Examination Survey; PFAS, per- and polyfluoroalkyl substances; PFOS, perfluorooctane sulfonic acid.
[a]p for interaction was calculated by log-likelihood ratio test.

beyond those included in the models could also affect the accuracy of the observed trends.[60] Accordingly, we may have underestimated the associations due to a lower contrast between extreme tertiles of PFAS/PFOS exposure. Third, a relatively small number of deaths from some specific causes make it infeasible to analyze deaths attributed to other causes. Given that we used mortality as an outcome, our results do not reflect the association between PFAS exposure and nonfatal heart disease or cancer. Although we found significant associations between PFAS mixture/PFOS exposure and all-cause mortality, deaths from causes likely not associated with PFAS mixture/PFOS exposure, such as accidents, were also included in the analysis. In addition, serum PFAS concentrations are measured only at the time when subjects were enrolled, so these data cannot reflect the change of exposure levels during the follow-up period. Last, but also important, using HRs derived from the follow-up data of NHANES participants from 1999–2014 to 2015 cannot accurately reflect the HRs for participants from NHANES 2015–2018, so the population attributable fractions for 2015–2018 are only rough estimates. Considering the decline of the PFOS exposure levels in the entire U.S population, HRs for participants from NHANES 2015–2018 would be lower than those for participants from NHANES 1999–2014, so we can conclude that the real population attributable fractions for 2015 to 2018 would be no larger than the estimated values in this study.

In conclusion, we observed a positive association between PFAS mixture exposure and mortality among U.S. adults. Results also suggest that PFOS, not PFOA, contributed in large part to the strength of the PFAS–mortality association, especially for women and people without diabetes. The decline of PFOS exposure levels in the United States reduced the number of deaths associated with PFOS from 1999 to 2018.

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